

The Acrosome and Its Differentiation during Spermiogenesis in *Halocynthia roretzi* (Ascidacea, Tunicata)

MAKOTO FUKUMOTO¹ and TAKAHARU NUMAKUNAI²

¹Biological Institute, College of General Education, Nagoya City University,
Mizuho-ku, Nagoya 467, Japan and ²Marine Biological Station,
Tohoku University, Asamushi, Aomori 039-34, Japan

ABSTRACT—The spermatozoa of *Halocynthia roretzi* have architectural features characteristic of ascidian spermatozoa that have been previously described. They have an elongated head (approximately 5.5 μm in length) and a single mitochondrion which is closely applied laterally to the nucleus. They lack a midpiece. An acrosome is present at the apex of the sperm head. The acrosome of *H. roretzi* spermatozoa is a depressed sphere, approximately $125 \times 125 \times 40$ nm.

At an early stage of spermiogenesis a vesicle (about 50 nm in diameter) appears in a blister at the apex of each spermatid. During spermiogenesis this vesicle becomes larger (approximately 100 nm in diameter). The vesicle in the blister contains moderately electron-dense material located along the vesicle membrane. At the completion of spermiogenesis the vesicle is most probably flattened by the elongating nucleus and transforms into an acrosome.

INTRODUCTION

Recent studies have shown that the spermatozoa of ascidians have an acrosome(s), albeit a small one [1, 5, 9]. In spite of various studies on fertilization in *H. roretzi*, there is still a debate over whether or not an acrosome is present in this species.

In *Ciona intestinalis*, the morphological changes in sperm associated with fertilization are becoming clear. An acrosome reaction occurs via vesiculation on the surface of the chorion or after penetration through the chorion. In the perivitelline space, apical processes protrude from the apex of the sperm head. Gamete fusion occurs between these apical processes and egg plasmalemma, resulting in the incorporation of the sperm head into the egg via its anterior region [6–8].

In order to develop a satisfactory understanding of the general mechanism of ascidian fertilization, further studies on morphological aspects of fertilization in other ascidian species are indispensable.

In this paper, the authors describe the acrosome and its differentiation during spermiogenesis as a preliminary step in the analysis of morphological aspects of fertilization in *H. roretzi*.

MATERIALS AND METHODS

The solitary ascidian, *Halocynthia roretzi* (Type C)[13, 14] was collected near Asamushi Marine Biological Station, Aomori, Japan.

For TEM observations on spermiogenesis and on the differentiated spermatozoa, the testes and the sperm duct were prepared for electron microscopy according to a method described by Fukumoto [2]. Electron micrographs were taken on a Hitachi H-300 electron microscope operated at 75 kV.

RESULTS

The acrosome in Halocynthia roretzi sperm

A fully differentiated spermatozoon of *H. roretzi* is approximately 40 μm length. It consists of a head and a tail. It lacks a midpiece. The head (approximately 5.5 μm long) contains an elon-

Accepted July 27, 1992

Received June 12, 1992

¹ To whom reprint request should be addressed.

gated nucleus and a single mitochondrion which flanks the nucleus (Fig. 1). An acrosome is present at the apex of the head (Fig. 1, arrow).

The fully differentiated acrosome is approximately $125 \times 125 \times 40$ nm (Figs. 2, 3 and 4). At the region where the acrosome is located, the inner and outer nuclear membranes are in extremely close contact with each other and form a "pedestal" for the acrosome (Figs. 2, arrow and 3).

Acrosome Differentiation during Spermiogenesis

The early spermatid is spherical and contains a round nucleus and a single mitochondrion. The chromatin exists as thin strands which are present throughout the nucleus. A fairly well developed Golgi apparatus with its associated vesicles is present in the cytoplasm (Fig. 5, arrow). The vesicle contains moderately electron-dense material (Fig. 6).

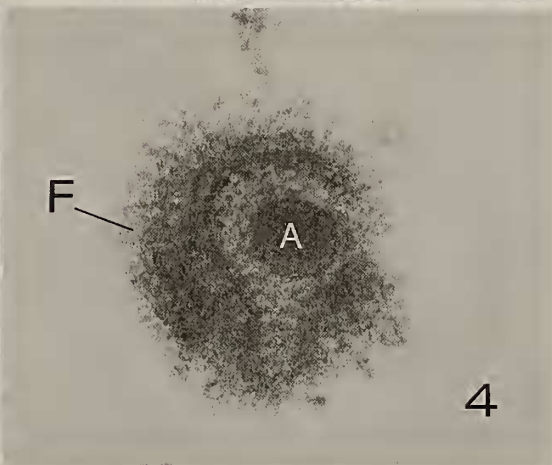
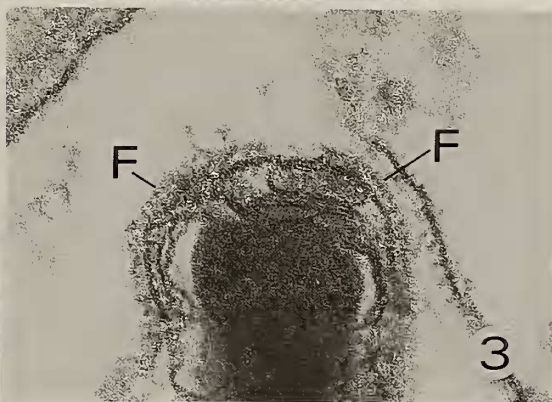
In spermatids mid-way through spermiogenesis, the nucleus has undergone some antero-posterior elongation. The future apex of the sperm corresponds to the blister (Fig. 7, arrow) and the future proximal end of the sperm head corresponds to the region where the flagellum extends out. During this stage the chromatin strands begin to become

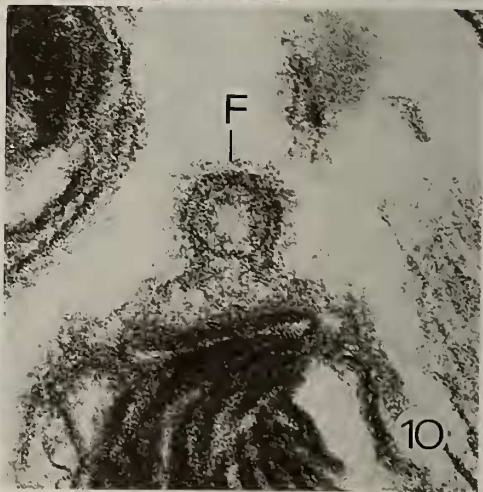
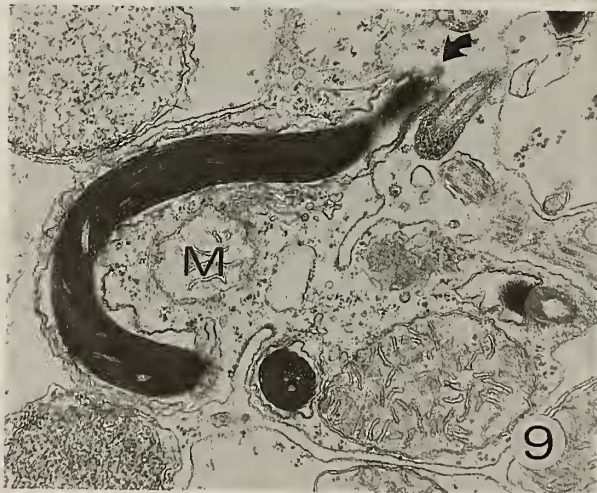
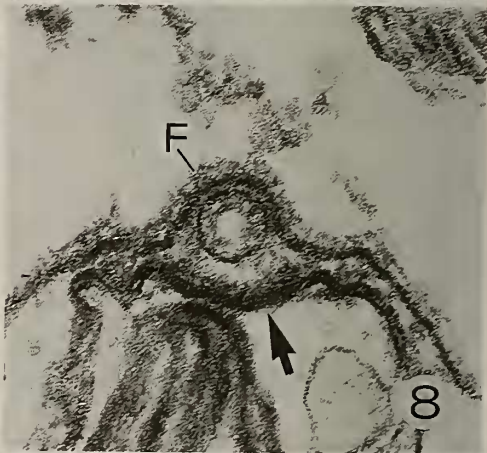
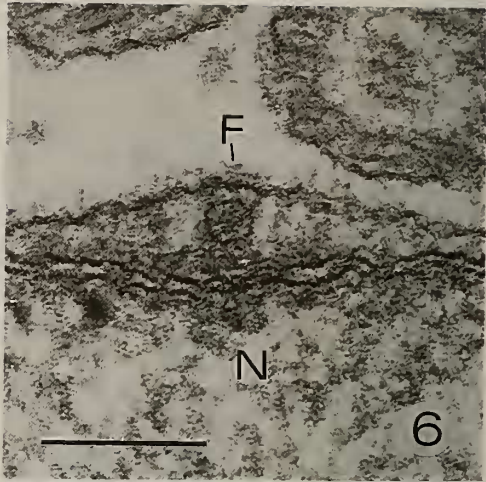
oriented parallel to the antero-posterior axis of the spermatid (Fig. 7). At this stage of spermiogenesis, the blister protrudes out from the apex of the sperm head. Moderately electron-dense material in the vesicle is located along the vesicle membrane (Fig. 8).

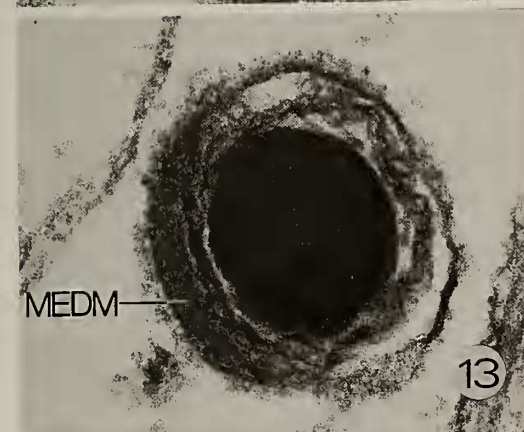
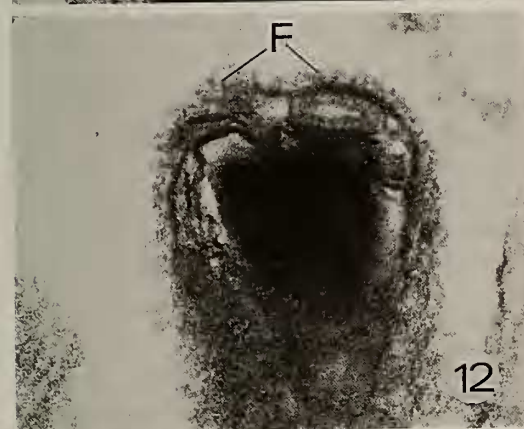
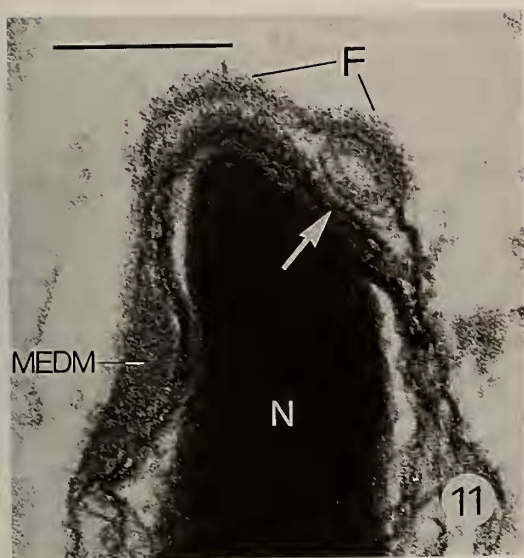
In spermatids at later stages of spermiogenesis, the nucleus further elongates antero-posteriorly in a helical configuration. This morphological change of the nucleus is accompanied by a change in the arrangement of the chromatin strands in the nucleus; chromatin strands which were arranged parallel to one another along the longitudinal axis of the nucleus coil up in a definite pattern (Fig. 9). A blister continues to be present in the anterior region (Fig. 9, arrow). A vesicle is present in a blister (Fig. 10). The nuclear membranes at the blister form an indentation, in which both the inner and the outer nuclear membranes are in close contact with each other (Figs. 8 and 10). The plasmalemma enclosing the blister is decorated by fuzzy material on its external surface (Figs. 8 and 10).

At the completion of spermiogenesis, the vesicle in the blister becomes a depressed sphere (Figs. 11 and 12) and transforms into an acrosome. This

- FIG. 1. Sagittal section through the head of a differentiated sperm. An acrosome (arrow) is present at the apex between the plasmalemma and the nuclear membranes. Fuzzy material (F) which decorates the outside of the plasmalemma enclosing the apex is thicker than it is on other parts of the head. M, mitochondrion; N, nucleus. The bar indicates $1 \mu\text{m}$.
- FIGS. 2 and 3. Sagittal and cross sections through the apex of a differentiated sperm head, respectively. An acrosome is present between the plasmalemma and nuclear membrane, where the inner and the outer nuclear membranes make close contact with each other to form a "pedestal" (arrow) for the acrosome. F, fuzzy material. The bar indicates 200 nm, which is also applicable to the Figs. 3 and 4.
- FIG. 4. Frontal slightly oblique section through the apex of the head. An acrosome (A) is recognized as a sphere in the central region of the pear-shaped pedestal. F, fuzzy material.
- FIG. 5. An early spermatid in which a blister is present (arrow). G, Golgi complex; M, mitochondrion; N, nucleus. The bar indicates $2.5 \mu\text{m}$ (This is also the magnification for Figs. 7 and 9).
- FIG. 6. Enlargement of the blister region. A vesicle which contains electron-dense material is present in the blister. Fuzzy material covers the outside of the plasmalemma enclosing the blister. F, fuzzy material; N, nucleus. The bar indicates 200 nm (This is also the magnification for Figs. 8 and 10).
- FIG. 7. Longitudinal section through a spermatid at a middle stage of spermiogenesis. The nucleus has begun to elongate antero-posteriorly. A blister (arrow) is present at the apex. M, mitochondrion; N, nucleus.
- FIG. 8. Enlargement of a blister region in a spermatid midway through spermiogenesis. A vesicle with a central electron lucent region is present in the blister. The inner and the outer nuclear membranes are in close contact with each other (arrow). Fuzzy material (F) decorates outside of the plasmalemma enclosing the blister.
- FIG. 9. Longitudinal section through a spermatid at a late stage of spermiogenesis where the elongated nucleus has a helical configuration. A blister (arrow) is present at the apex. M, mitochondrion.
- FIG. 10. Enlargement of a blister region in a late stage spermatid. The blister projects more than that at early stages of spermiogenesis. A vesicle is present in the blister. Fuzzy material (F) decorates outside of the plasmalemma enclosing the blister.







FIGS. 11 and 12. Sagittal and transverse sections through the apex of the head of a sperm that has almost completed maturation. The vesicle in the blister becomes an ellipsoid. Where the vesicle is

morphological change of the vesicle most likely occurs as a consequence of the elongation of the nucleus. Moderately electron-dense material (MEDM) is present in the space between the plasmalemma and the nuclear membranes (Fig. 11). A cross section at the level of the MEDM reveals that this material is located at one side of the sperm head (Fig. 13) below the acrosome (Fig. 11).

DISCUSSION

During acrosome differentiation in other animal species, proacrosomal vesicles derived from Golgi complex(es) coalesce to form one acrosomal vesicle. In ascidians, a similar process has been reported. In *Pyura haustor* and *Styela plicata*, two vesicles usually appear in a blister at the apex of these spermatids in an early stage of spermiogenesis. They fuse to form a single acrosome-like structure in the apex of differentiated spermatozoa [3]. In *Molgula manhattensis*, at least three or four vesicles which are moderately electron-dense appear in the blister at the apex of spermatid in early stages of spermiogenesis. They fuse with each other to form an acrosomal vesicle [4]. In *H. roretzi*, in most cases, one vesicle appears in the blister of young spermatids. The vesicle becomes larger during the course of spermiogenesis (compare Figs. 6 and 8). This probably occurs via the coalescence of smaller vesicles. These findings are consistent with reports on acrosome differentiation in other animal species. With respect to the origin of the vesicle in the blister, no direct evidence has been found to show that it is derived from the Golgi apparatus. In view of the fact, however, that early spermatids have a fairly well-developed Gol-

located, the inner and the outer nuclear membranes come into close contact with each other to form a "pedestal" (arrow) for the vesicle. Moderately electron-dense material (MEDM) is present in the space between the plasmalemma and the nuclear membranes near the apex. Fuzzy material (F) covers the outside of the plasmalemma enclosing the apex. N, nucleus. The bar indicates 200 nm, which is also applicable to Figs. 12 and 13.

FIG. 13. Transverse section through the head at the level of the MEDM. The MEDM occupies almost half of the space around the nucleus.

gi apparatus and Golgi-derived vesicles, it seems safe to assume that they are derived from Golgi vesicles.

Recently, Lambert and Koch [12] have argued that the vesicles in ascidian spermatozoa should be called 'apical vesicles' until criteria for their positive identification as acrosomes can be more closely met. However, if a vesicle(s) is present in the proper location for an acrosome, this would be one kind of evidence for the existence of an acrosome, because acrosomes are anteriorly located vesicles in the sperm of other animals. Furthermore the vesicle in ascidian spermatozoa is not an oddity which is only found in one or two species, but a feature of all species examined in the Orders of Pleurogonan and Enterogonan ascidians [5, 9]. These are two good reasons for applying the term "acrosome" to these vesicles. We can conclude that spermatozoa of *H. roretzi* have an acrosome as has been confirmed in other ascidian species [9].

Recently it has been found that caffeine or theophylline induces morphological changes in the acrosome of *Ciona intestinalis* spermatozoa. These morphological changes have been referred to as an acrosome reaction. The acrosome reaction occurs by fusion between the acrosomal outer-membrane and the plasmalemma enclosing the acrosome. The fusion seems to proceed along the peripheral margin of the acrosome, resulting in vesiculation [8]. Morphological studies on the acrosome reaction in *H. roretzi* are now in progress.

In *H. roretzi*, the egg is enclosed by a relatively thick acellular chorion (vitelline coat). For successful gamete fusion, spermatozoa must pass through the chorion. Sperm might utilize chorion lysin(s) in order to pass through the chorion. Indeed, there is evidence that spermatozoa of *H. roretzi* contain proteases that act as lysins [10, 15]. However, it is not clear where the lysins are located in spermatozoa. Recent studies on fertilization in *H. roretzi* reveal that spermatozoa can pass through the chorion (vitelline coat) with an intact acrosome and have loose their acrosome in the perivitelline space (Fukumoto, in preparation). This suggests that chorion lysin(s) are associated with the surface of the plasma membrane of sperm head. The chemical nature and the precise role of the acrosomal substance remain

obscure.

In *H. roretzi*, Kubo *et al.* [11] reported that an acrosome was formed from Golgi-derived vesicles midway through spermiogenesis. They speculated that during further differentiation this acrosome was translocated to the space between the plasmalemma and the nuclear membranes near the apex of the sperm head, where it was recognized as an accumulation of moderately electron-dense material (MEDM). Although the MEDM is not a vesicular structure, they considered it homologous to an acrosome. However, they concluded that the acrosome had disappeared before the completion of spermiogenesis, because they could not find an acrosome in mature spermatozoa. In this study an MEDM was seen in the same region of spermatids as has been described by Kubo *et al.* [11] during spermiogenesis (Figs. 11 and 13). In contrast to their descriptions, the MEDM is not homologous to an acrosome (Fig. 11). The precise role and fate of the MEDM remains unknown at present. Although Kubo *et al.* [11] found the blister at the anterior tip of spermatid, they did not find the vesicular "precursor of the acrosome" in the blister and the acrosome at the apex of the differentiated sperm head, which are described here.

ACKNOWLEDGMENTS

The authors are grateful to Professor Gary Freeman, of the University of Texas at Austin, for his valuable suggestions and for reading the manuscript. The author (M.F.) wishes to thank Professor Kenji Osanai, the director of the Asamushi Marine Biological Station, for facilities. We also wish to thank Mr. S. Tamura and Mr. M. Yashio, of Asamushi Marine Biological Station, for their help in collecting materials.

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- 1 Cloney RA, Abbott LC (1980) The spermatozoa of ascidians: acrosome and nuclear envelope. *Cell Tiss Res* 206: 261-270
- 2 Fukumoto M (1981) The Spermatozoa and Spermiogenesis of *Perophora formosana* (Ascidia) with Special Reference to the Striated Apical Structure and the Filamentous Structures in the Mitochondrion. *J Ultrastruct Res* 77: 37-53

- 3 Fukumoto M (1983) Fine Structure and Differentiation of the Acrosome-like Structure in the solitary Ascidian, *Pyura haustor* and *Styela plicata*. *Develop Growth Differ* 25: 503–515
- 4 Fukumoto M (1985) Acrosome Differentiation in *Molgula manhattensis* (Ascidacea, Tunicata). *J Ultrastruct Res* 92: 158–166
- 5 Fukumoto M (1986) The acrosome in ascidians. I. Pleurogona. *Int J Invert Reprod Dev* 10:335–346
- 6 Fukumoto M (1988) Fertilization in ascidians: apical processes and gamete fusion in *Ciona intestinalis* spermatozoa. *J Cell Sci* 89: 189–196
- 7 Fukumoto M (1990) Morphological aspects of ascidian fertilization: Acrosome reaction, apical processes and gamete fusion in *Ciona intestinalis*. *Invert Reprod Develop* 17: 147–154
- 8 Fukumoto M (1990) The Acrosome Reaction in *Ciona intestinalis* (Ascidia, Tunicata). *Develop Growth Differ* 32: 51–55
- 9 Fukumoto M (1990) Review: Morphological Aspects of Ascidian Fertilization. *Zool Sci* 7: 989–998
- 10 Hoshi M, Numakunai T, Sawada H (1981) Evidence for participation of sperm proteases in fertilization of the solitary ascidian, *Halocynthia roretzi*: effects of protease inhibitors. *Dev Biol* 86: 117–121
- 11 Kubo M, Ishikawa M, Numakunai T (1978) Differentiation of apical structures during spermiogenesis and fine structure of the spermatozoon in the Ascidian *Halocynthia roretzi*. *Acta Embryol Exp* 3: 289–295
- 12 Lambert CC, Koch RA (1988) Review: Sperm Binding and Penetration during Ascidian Fertilization. *Develop Growth Differ* 30: 325–336
- 13 Numakunai T, Hoshino Z (1973) Biology of the ascidian, *Halocynthia roretzi* (Drasche), in Mutsu Bay. I. Differences of spawning time and external features. *Bull Mar Biol Stat Asamushi, Tohoku Univ* 14: 191–196
- 14 Numakunai T, Hoshino Z (1974) Biology of the ascidian, *Halocynthia roretzi* (Drasche), in Mutsu Bay. II. One of the three types which has the spawning season and the time different from two others. *Bull Mar Biol Stat Asamushi, Tohoku Univ* 15: 23–27
- 15 Sawada H (1990) Sperm penetration through the vitelline coat in ascidian. In "Advances in Invertebrate Reproduction 5" Ed by M Hoshi, O Yamashita, Elsevier Science Publishers BV (Biomedical Division), pp 111–116